SUPPLEMENTARY INFORMATION

Leukocyte Adhesion Deficiency-III (LAD-III) is caused by mutations in the adhesion protein KINDLIN-3

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SUPPLEMENTARY METHODS

Flow cytometry Leukocytes (5 x10^5) were incubated for 20 min at 4 °C in 50 μl of PBS with 0.2% BSA containing primary mAb at optimal dilution as described previously[1]. Bound mAb was detected by incubation with FITC-conjugated goat anti-mouse IgG (Sigma) for 20 min at 4 °C and analysed by a FACS Calibur flow cytometer (BD Biosciences).

Immunoblotting Cells (5 x 10^7) were lysed in 0.5 ml buffer (1% TritonX-100, 50 mM Tris-base pH 7.5, 150 mM NaCl and complete protease inhibitor cocktail (Roche Diagnostics Ltd.). Cell lysate proteins were reduced in 2 x Laemmli buffer and separated using pre-cast 4–12% SDS-PAGE gels (Invitrogen) or 7.5% SDS-PAGE gels, transferred to nitrocellulose (Hybond™-ECL™, GE Healthcare) or PVDF membrane (Immobilon-P, Millipore) and probed with various Abs as specified. Rabbit Ab was detected with goat anti-rabbit IgG-HRP (Dako), mouse mAbs with sheep anti-mouse Ig-HRP and ECL detection reagents (GE Healthcare).

Cell attachment assays Flat-bottom Immulon-1 96 well plates were coated with 100 μl ICAM-1Fc (3 μg ml^{-1}) or fibronectin (10 μg ml^{-1}) in PBS overnight at 4 °C and blocked with 2.5% BSA. Cells were labelled with 2.5 μM 2',7'-bis-(carboxyethyl)-5(6')-carboxyfluorescein (BCECF; Merck Chemicals) then washed into HBSS with 20 mM HEPES and plated at 2 x 10^5 cells per well (samples in triplicate). The cells were treated as indicated and incubated at 37 °C for 30 min. Non-adherent cells were removed by gentle washing and

adhesion quantified using a Cytofluor multiwell plate reader (PerSeptive Biosystems).

Suppl. Fig. 1

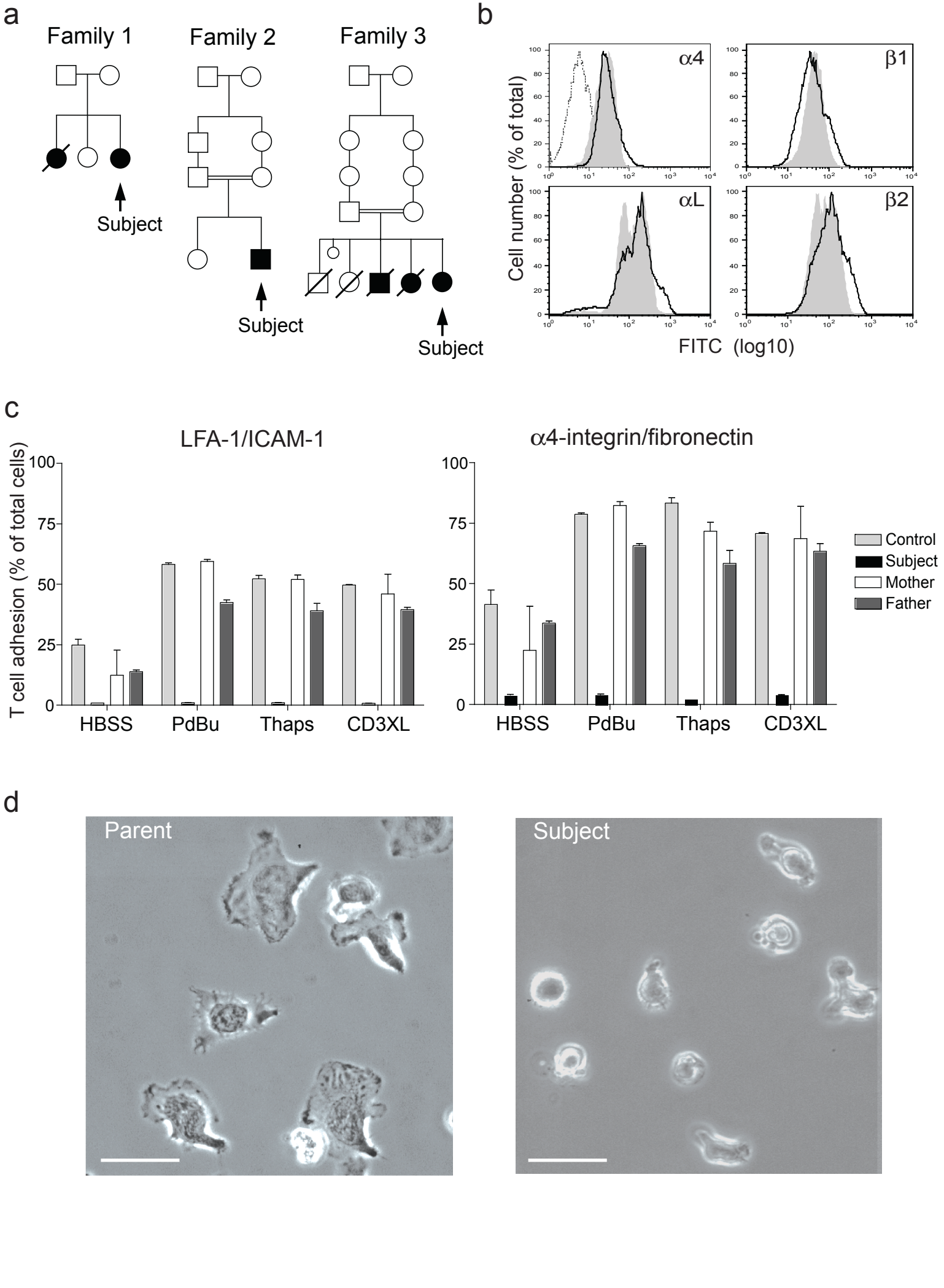

a
Family 1
Family 2
Family 3
Subject
Subject
Subject
b
α4
β1
αL
β2
Cell number (% of total)
FITC (log10)
c
LFA-1/ICAM-1
α4-integrin/fibronectin
T cell adhesion (% of total cells)
HBSS
PdBu
Thaps
CD3XL
Control
Subject
Mother
Father
d
Parent
Subject

Figure 1. LAD-III patient genealogy and characterization of a new LAD-III subject. (**a**) The pedigrees of the three LAD-III patients and their families are shown. The clinical features of families one (Maltese) and two (Turkish) have been reported previously[1,2]. The parents in family three are the grandchildren of Turkish sisters. A double line indicates the level of known consanguinity. Female, circle; male, square. A forward line in the subject generation indicates that the individual is deceased; (**b**) FACS analysis showing equivalence of the expression levels of the integrin subunits α4, β1, αL and β2 between family three LAD-III patient and control T lymphoblasts (control cells -grey fill; subject cells -dark line; representative mAb negative control-dashed line); (**c**) Adhesion of family three T lymphoblasts to LFA-1 ligand ICAM-1 and α4β1/α5β 1 ligand fibronectin following "inside out" signalling mediated by phorbol ester (PdBu), Ca2+ mobiliser thapsigargin (Thaps); or CD3 mAb crosslinking of the T cell receptor/CD3 complex (CD3XL) (*n* = 2). The LAD-III T lymphoblasts display a failure to attach following "inside out signals". LAD-III T lymphoblasts also fail to attach to ICAM-1 when stimulated with SDF-1(CXCL12) unlike the control T lymphoblasts (data not shown). (**d**) Images of T lymphoblast adhesion to ICAM-1. The family 3 parent's cells spread as expected, whereas the family three subject's cells were lightly attached and failed to spread. Scale bar = 10 μm.

References

1. McDowall, A. et al. A novel form of integrin dysfunction involving beta1, beta2, and beta3 integrins. J. Clin. Invest. 111, 51-60 (2003).
2. Kuijpers, T.W. et al. Natural history and early diagnosis of LAD-1/variant syndrome. Blood 109, 3529-37 (2007).

Suppl. Fig. 2.

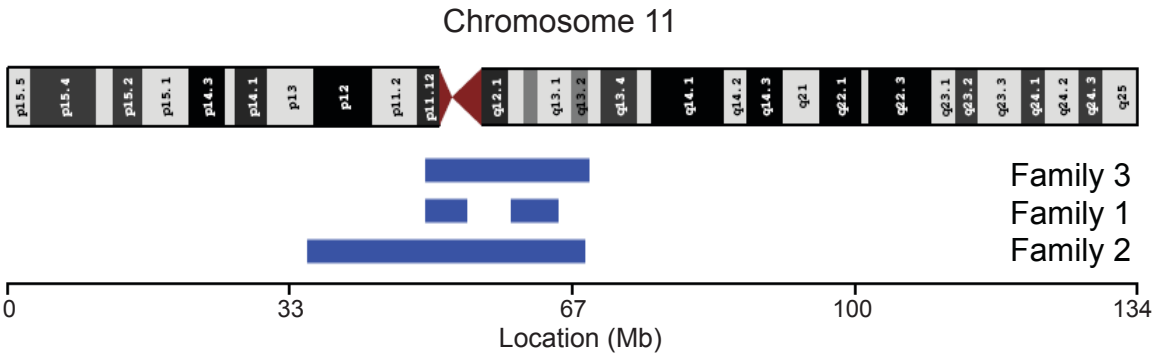
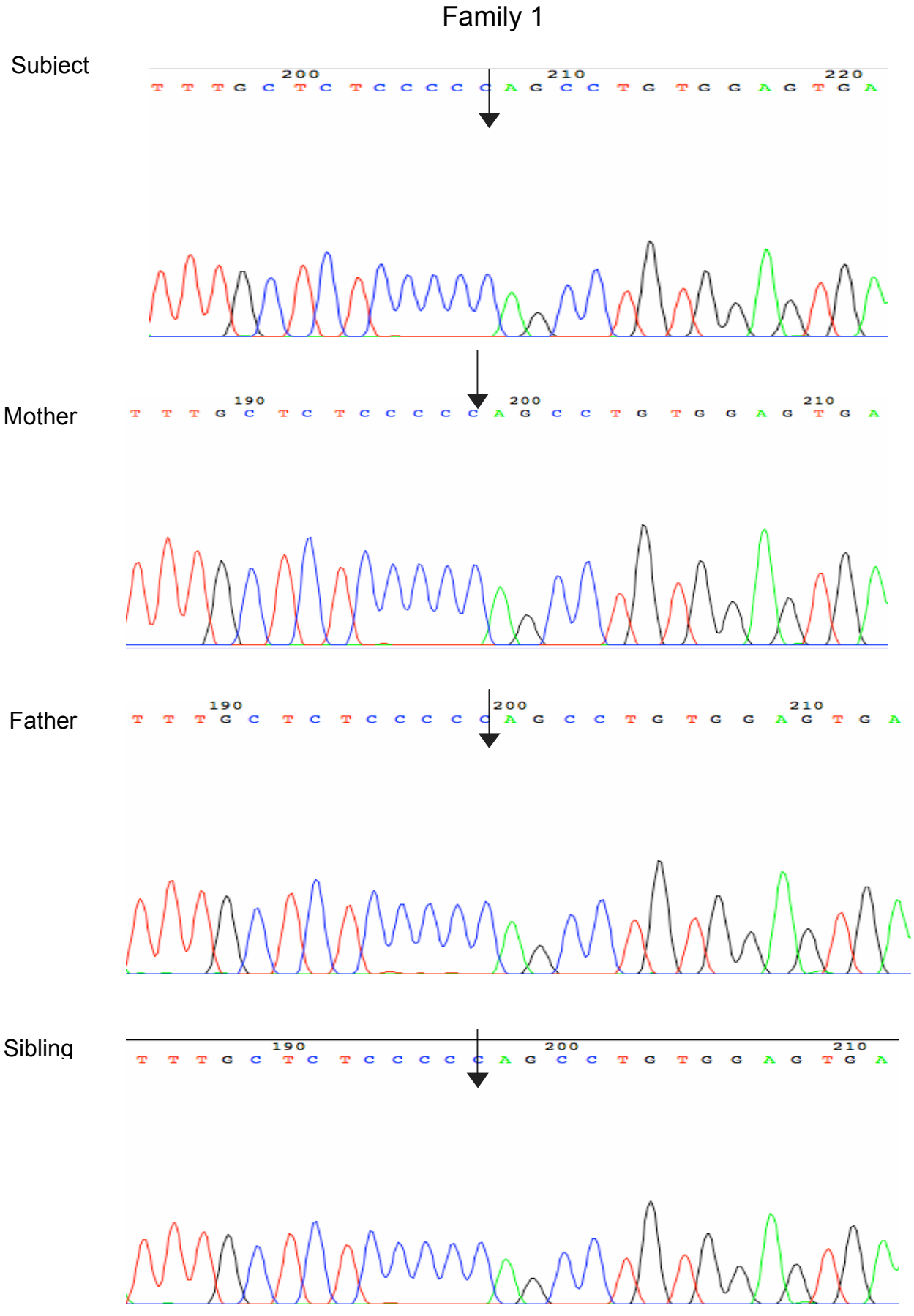


Figure 2. Homozygosity mapping. The display from the program http://uk-lif-lbio02.crnet.org/~cazier01/ indicates the largest region of homozygosity shared among affected individuals (chr 11: 60.6 Mb to 65.3 Mb; purple bars) and absent in unaffected family members from the three kindreds. Data are from Affymetrix 500 K SNP arrays with a mis-call tolerance of 1%. There were no additional shared areas in the whole genome other than at centromere regions with larger than 1 Mb homozygosity in all three subjects. In the absence of knowledge of allele frequencies in the almost certainly inbred populations under study, this relatively simple display method does not take SNP information content into account. Hence centromeric regions (red) with low homozygosity are often shown falsely, as here, as being identical by descent.

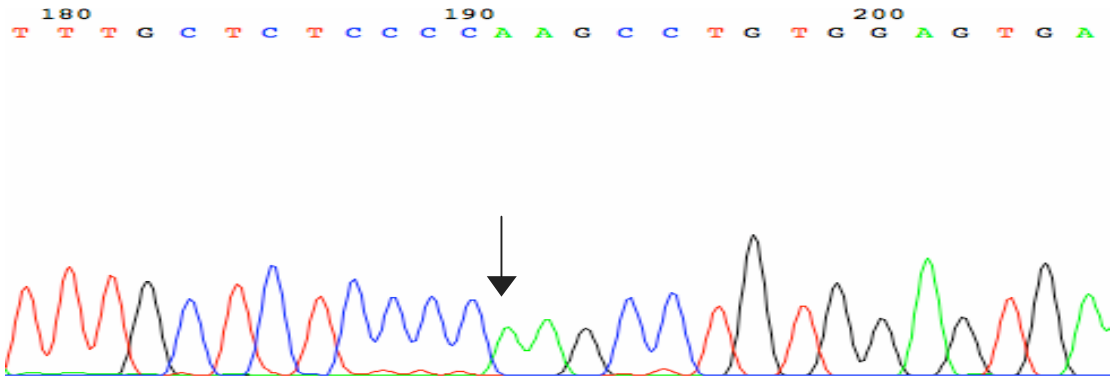
Suppl. Fig. 3

CALDAG-GEF1 (RASGRP2): sequence surrounding the C/A base change at the splice junction (exon 16)

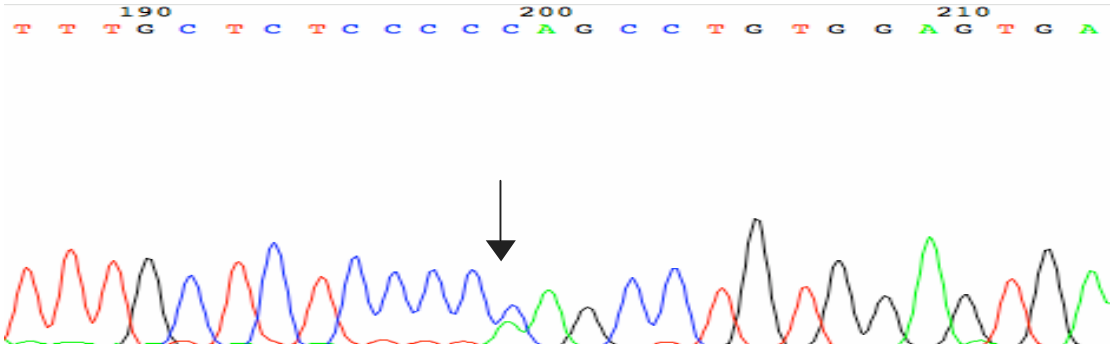


Family 2

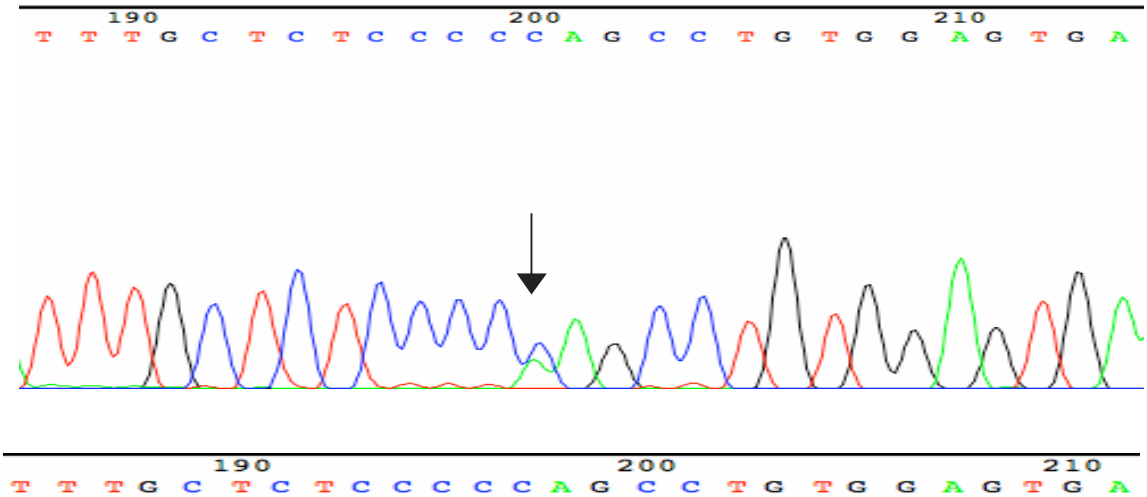
Subject



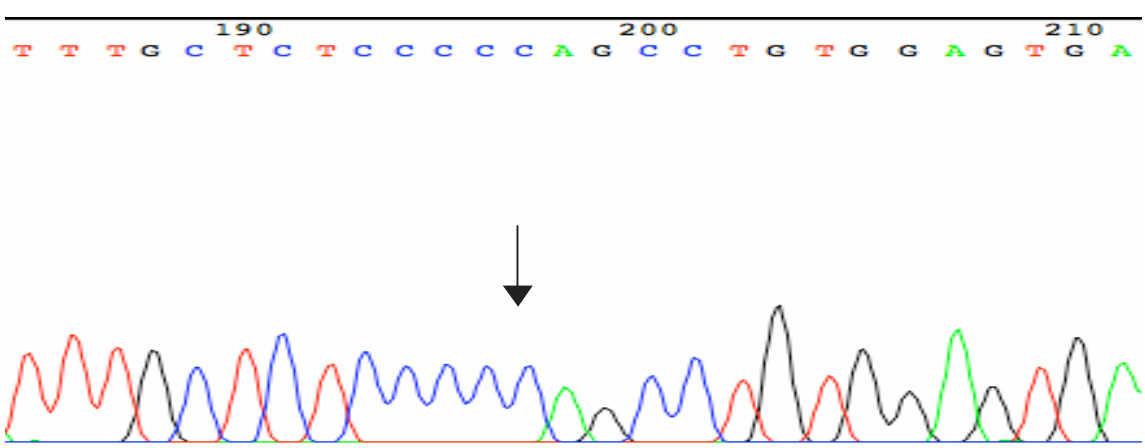
Mother



Father



Sibling



Family 3

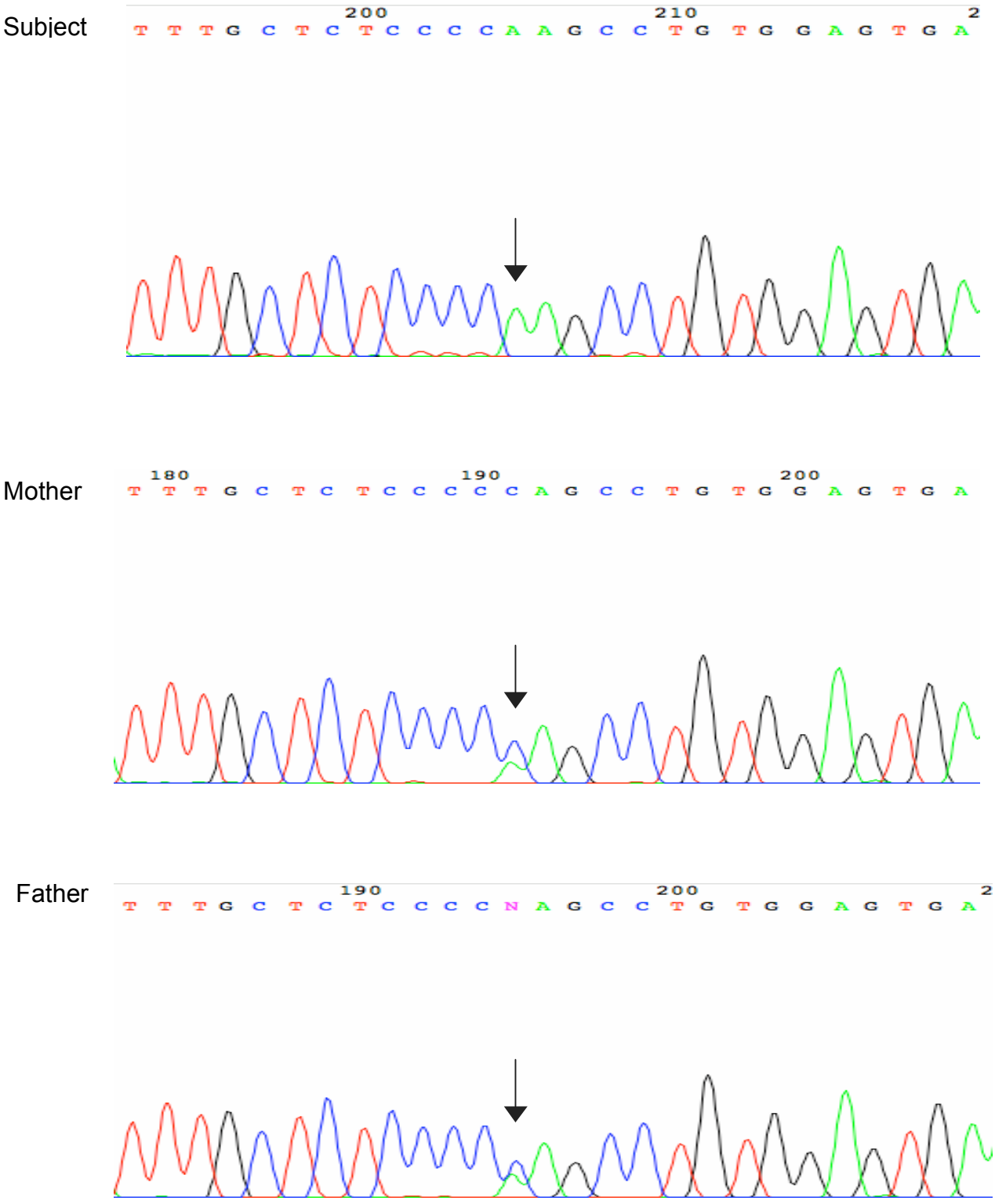


Figure 3. *CALDAG-GEF1 (RASGRP2)* sequence. The sequence of the genomic DNA surrounding the C/A base change at exon 16 in *CalDAG-GEF-1* (see arrow) is shown for all LAD-III subjects and their relatives. The base change is present in the Turkish LAD-III subjects and their relatives (family two -but not sister- and family three) and not in Maltese family one.

Suppl. Fig. 4

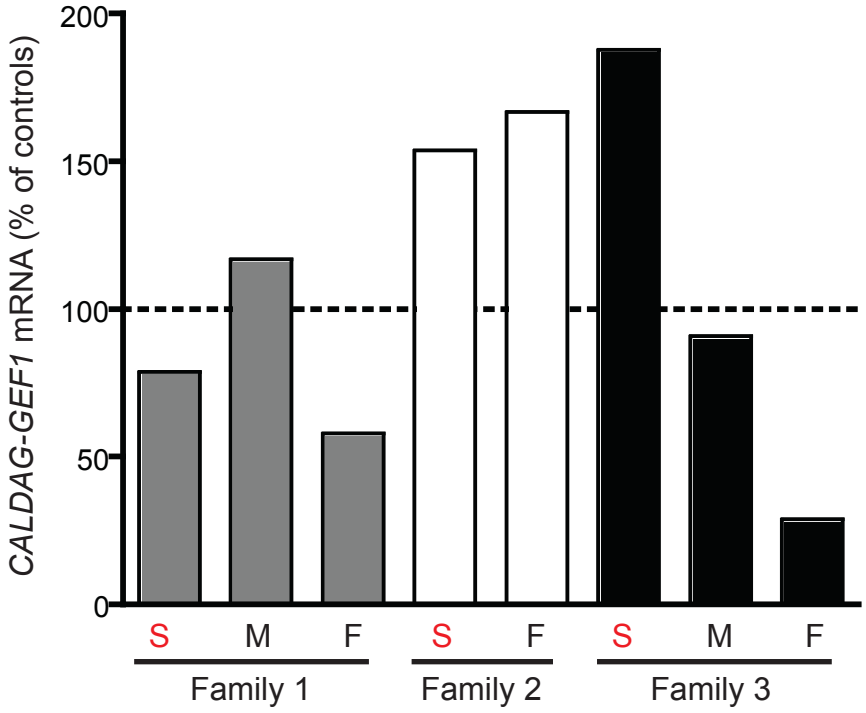
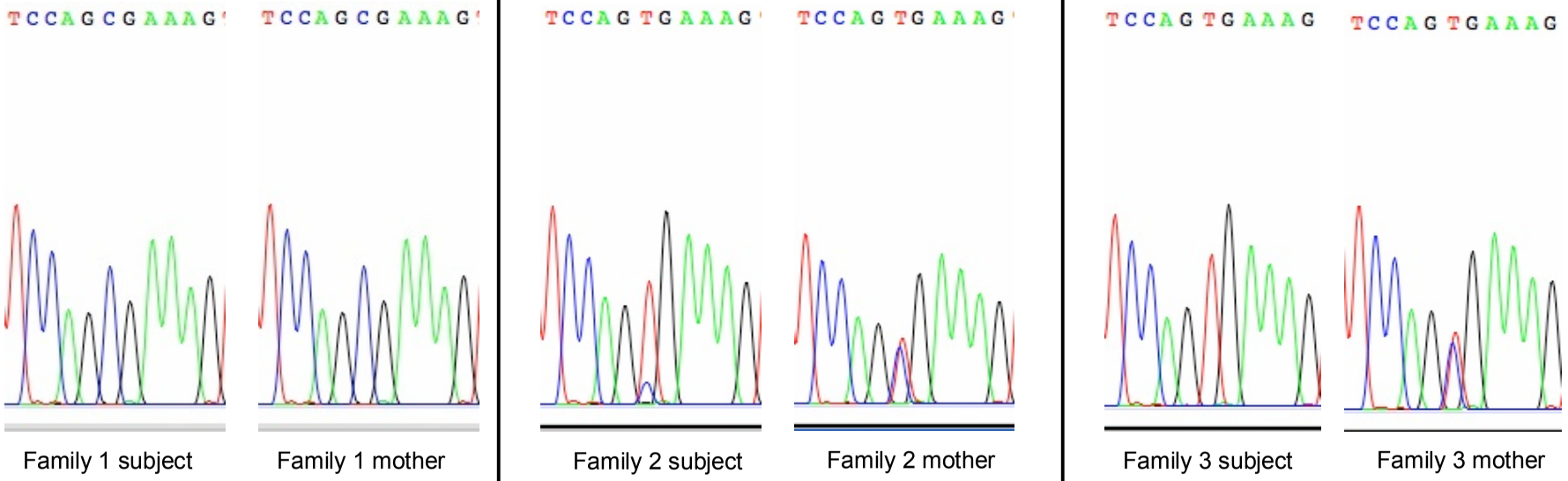
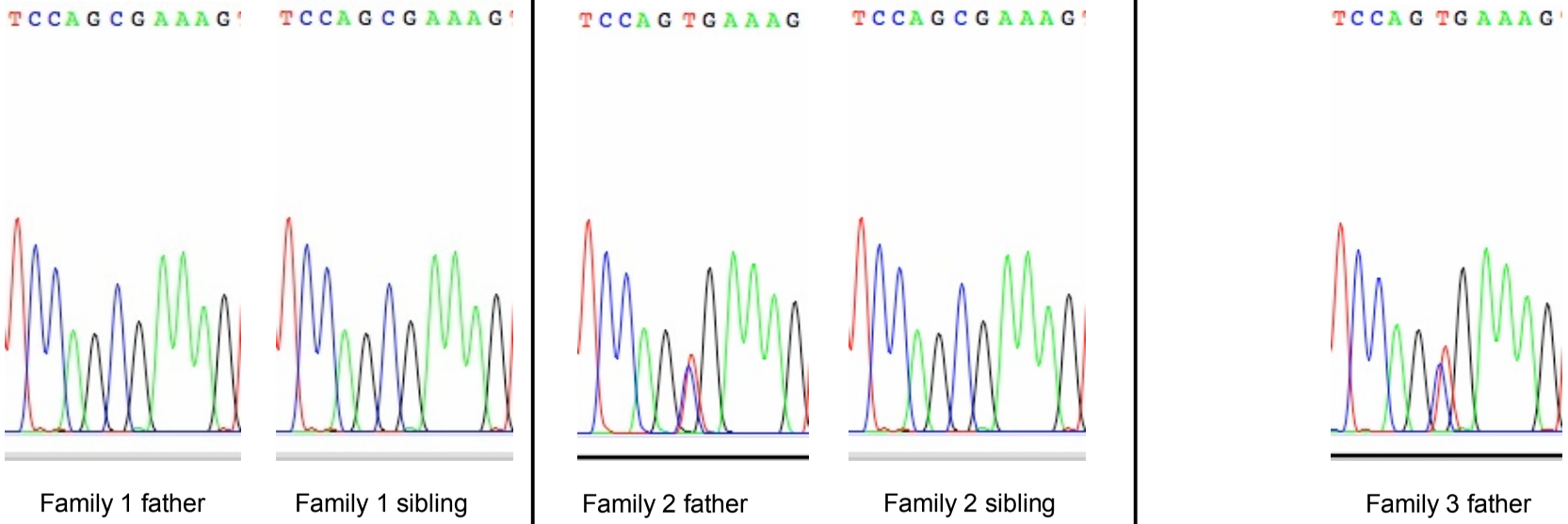


Figure 4. Analysis of CALDAG-GEF1 (RASGRP2) mRNA. Quantification of *CALDAG-GEF1* mRNA levels by TaqMan analysis of subjects (S) and their relatives (M=mother; F=father) compared with two normal control EBV transformed cell lines (*n* = 2–4 for each family). The Taqman prbes were located in CALDAG-GEF1 exons 8 nd 9.

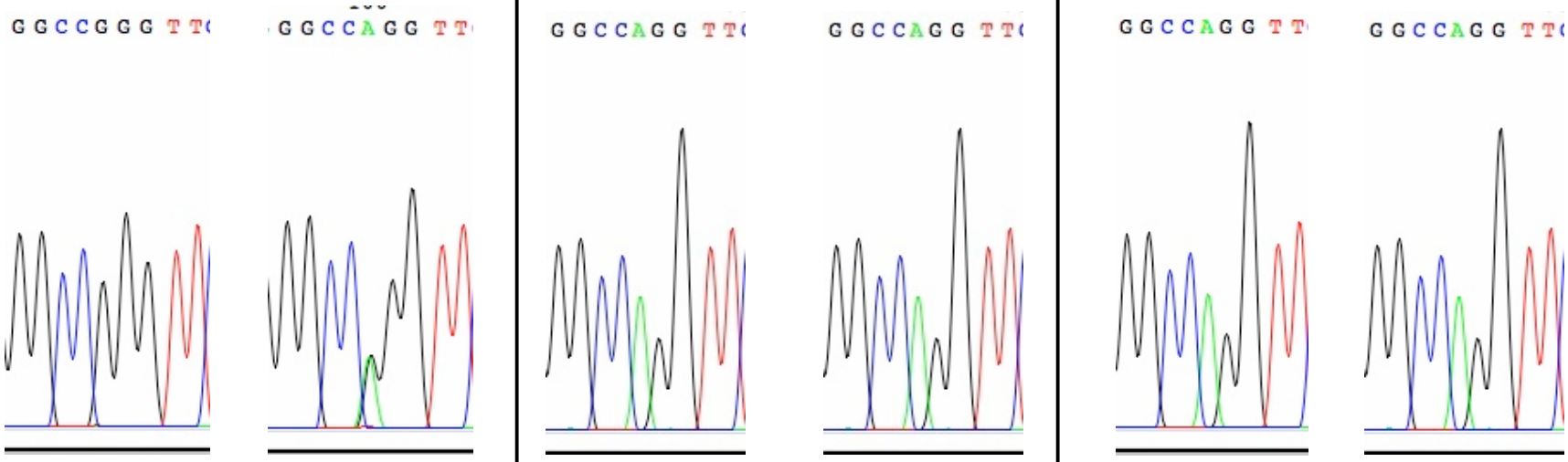
Suppl. Fig. 5

KINDLIN-3: sequence surrounding the C>T mutation (exon 12)

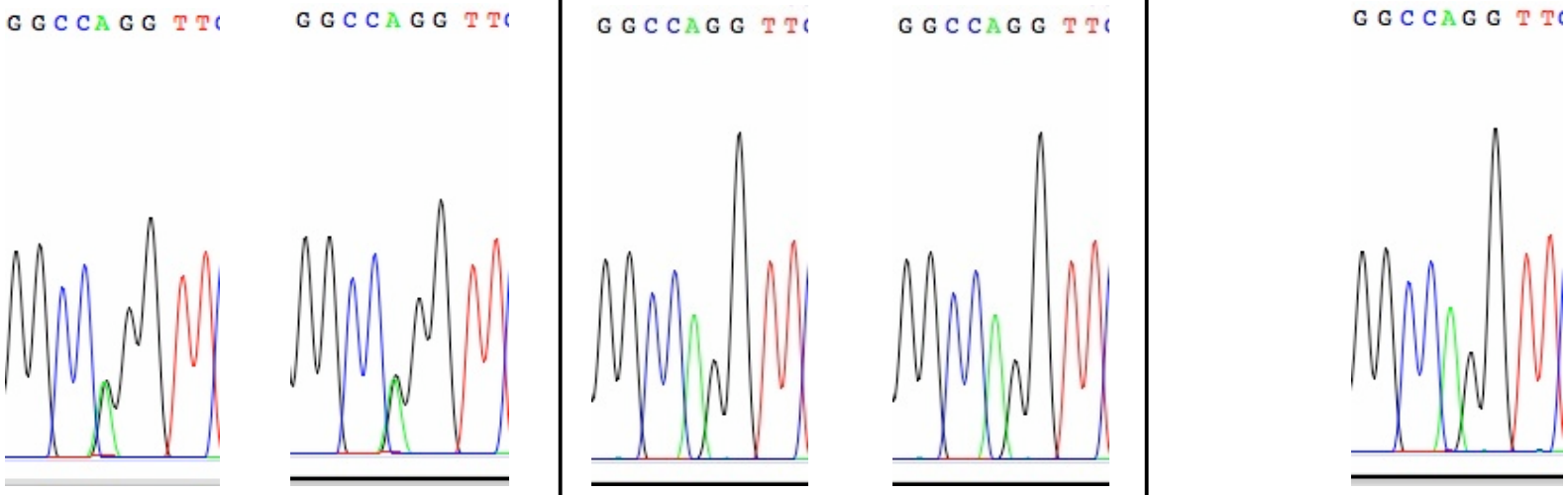




KINDLIN-3: sequence surrounding the splice acceptor site A>G mutation (exon 14)



Family 1 subject | Family 1 mother | Family 2 subject | Family 2 mother | Family 3 subject | Family 3 mother



Family 1 father | Family 1 sibling | Family 2 father | Family 2 sibling | Family 3 father

Figure 5. *KINDLIN-3* (*FERMT3)* sequences: exon 12 and exon 14 splice acceptor site mutations. The sequences of the genomic DNA surrounding the *KINDLIN-3* C>T mutation in exon 12 found in the Turkish families and the A>G mutation associated with the exon 14 splice acceptor site in the Maltese family are shown for all LAD-III subjects and their relatives.

SUPPLEMENTARY VIDEO 1

GFP-expressing LAD-III B cells of the subject from Turkish family three migrating on ICAM-1. These cells have dynamic membranes but are not motile. Each frame = 1/10 s representing 15 s real time.

SUPPLEMENTARY VIDEO 2

CALDAG-GEF-1–expressing LAD-III B cells of the subject from Turkish family three migrating on ICAM-1. These cells have dynamic membranes, are not motile and resemble the cells expressing only GFP (video 1). Each frame = 1/10 s representing 15 s real time.

SUPPLEMENTARY VIDEO 3

Kindlin-3–expressing LAD-III B cells of the subject from Turkish family three migrating on ICAM-1. Expression of Kindlin-3 renders these cells motile almost to the same extent as the parent's cells in Video 4. Each frame =1/10 s representing 15 s real time.

SUPPLEMENTARY VIDEO 4

GFP-expressing B cells of the parent of family three migrating on ICAM-1. Each frame =1/10 s representing 15 s real time.